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RESEARCH PAPER

Mutated *TP53* is a marker of increased *VEGF* expression: analysis of 7,525 pan-cancer tissues

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ABSTRACT

Anti-angiogenic therapies are an important class of anti-cancer treatment drugs. However, their efficacy is limited to certain tumors and would benefit from identifying a biomarker predictive of therapeutic response. *TP53* (tumor protein p53) is a tumor suppressor gene frequently mutated in cancer and implicated in cell-cycle regulation, apoptosis, and angiogenesis. Data from 7,525 unique tumor samples (representing 30 tumor cohorts) were retrieved from the TCGA database to analyze the relationship between *TP53*-mutation status and *VEGFA (vascular endothelial growth factor A)* expression. Univariate analyses were done using a Mann-Whitney univariate test or Fisher's exact test. Parameters with a p-value (p)≤0.1 in univariate analysis were selected for follow-up multivariate analyses, including *TP53*-mutation status, cancer cohorts, cancer subtypes, and *VEGFA* expression. Our analysis demonstrates statistically significant increases in *VEGFA* mRNA tissue expression in *TP53*-mutated adenocarcinomas (but not in squamous cancers) compared to *TP53* wild-type tumors. This association holds true in multivariate analyses and remains independent of *HIF-1α* and *MDM2* overexpression. Our findings provide additional evidence that *TP53* mutations are linked to the VEGF pathway, potentially offering insight into the mechanism behind increased sensitivity to anti-angiogenic therapies observed in some *TP53*-mutant tumors.

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Tumor suppressor protein p53; vascular endothelial growth factor A; biomarkers; genomics; multivariate analysis

Introduction

Personalizing therapy for cancer patients by pairing their tumor molecular profiles with "matched" treatments has demonstrated success in several clinical contexts.[1–3] However, continuing to identify biomarkers that predict therapeutic response is necessary to expand this approach. In particular, anti-angiogenic therapies (dugs which target the neo-vascularization process allowing tumors to self-sustain) would benefit from identification of a specific biomarker. This pharmacology class encompasses over ten approved antibodies or small molecule inhibitors that target the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) axis (Supplementary Figure S1). Indeed, one of the best-selling drugs in oncology is bevacizumab, a VEGF-A monoclonal antibody. Though indicated for use in a variety of cancers, such as renal cancer, colon cancer, non-small cell lung cancer (NSCLC) and glioblastoma, the impact on survival in non-selected patients is modest, and bevacizumab's approval in metastatic breast cancer was revoked in 2011 by the Food and Drug Administration (FDA).[4] Anti-angiogenic therapies are also expensive and have numerous side effects, including gastrointestinal perforation, hypertension, and hemorrhage.[5,6] Identifying specific parameters that predict response to anti-angiogenesis therapy may be used to separate patients likely to benefit from those that might be transferred to an alternative therapy.

TP53 (tumor protein p53) is a multifunctional tumor suppressor gene that is also intimately involved in the process of neo-vascularization, often through various inhibitory mechanisms.[7] For example, TP53 promotes degradation of the hypoxia-induced factor subunit α (HIF-1α) in the cell. HIF-1 serves as a key transcriptional activator of VEGF-mediated angiogenesis in response to oxygen deprivation.[8] The relationship between TP53 and HIF-1α is not yet fully clear, but TP53 appears to also affect angiogenesis via other pathways. One study demonstrated that TP53 inhibits VEGF expression through a pathway involving tumor protein 21 (p21) and retinoblastoma (Rb) in vitro.[9] Another study characterized the E2F transcription factor 1 (E2F1) and found an apparent relationship with TP53 to directly downregulate VEGF expression in a HIF-1α independent fashion.[10] A role for TP53 in angiogenesis has been established in multiple studies – in bone marrow stromal cells, transfection of mutant TP53 increased synthesis of VEGF and supported leukemia cell growth.[11] Angiogenesis is an area of interest in these disorders, as vascular development has been observed in lymph nodes and bone marrow in patients with various hematologic malignancies; treatment strategies are being explored in this area.[12] In a separate study demonstrating the reverse, the restoration of *TP53* expression in

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a population of previously altered-*TP53* cells was associated with decreased angiogenesis.[13]

Somatic mutations in the *TP53* gene are found in high rates across multiple cancers, such as colorectal, lung, and head and neck – even ranging up to over 85% in high-grade ovarian serous carcinoma.[14] Mutations in *TP53* are used as markers of clonality, recurrence and cancer prognosis,[15] but are often considered non-actionable by conventional therapies.

Recently, several studies have suggested that *TP53* mutation status may be predictive of clinical sensitivity to VEGF/VEGFR inhibitors in certain tumors. A recent analysis showed that *VEGFA* transcript expression correlated independently with *TP53* mutational status in patients with adenocarcinoma (but not squamous) NSCLC.[16] Additional studies have demonstrated statistically significant improvements in clinical outcomes (such as response rate, progression-free survival, and overall survival) among *TP53*-mutant patients treated with anti-VEGF or anti-VEGFR therapies, compared to *TP53*- wild-type populations.[17–20] The increased expression of *VEGFA* may explain the improved response to VEGF/VEGFR inhibitors in *TP53*-mutant populations, but to date, datasets have only interrogated a relatively small number of patients. Herein, we present an analysis of *TP53* mutation status and *VEGF/VEGFR* expression in a large pan-cancer cohort, using a collection of 7525 tumor samples from The Cancer Genome Atlas (TCGA).

Results

Somatic *TP53* mutations are frequent in human tumors

Of 10,011 samples available through the TCGA database, only 7,525 (30 tumor types) included available data on both *TP53* mutation status and VEGF pathway transcript expression estimates (Table 1). Analyses were performed on the full set of 7,525 samples (pan-cancer cohort) and/or by individual tumor types.

TP53 mutations were found in 35% of all samples. The most mutated cohorts, percentage-wise, were uterine carcinoma (50/56 samples, 89%), ovarian serous cystadenocarcinoma (210/245, 86%), and squamous NSCLC (145/178, 81%). In contrast, uveal melanoma (0/80, 0%), pheochromocytoma and paraganglioma (1/161, 1%), and thyroid carcinoma (3/397, 1%) contained the lowest percentage of *TP53*-mutated samples.

VEGF pathway biomarkers are differentially expressed in several subsets of *TP53*-mutated tumors

Table 2 presents the analysis of mRNA expression levels of key angiogenesis biomarkers, considering the presence or absence of a somatic *TP53* mutation.

When examining specific cancer cohorts, *TP53* mutations were associated with a significant increase of *VEGFA* mRNA expression levels in breast carcinoma, colon adenocarcinoma, and NSCLC adenocarcinoma, compared to *TP53* wild-type tumors (univariate analysis, breast carcinoma $p < .001$, colon adenocarcinoma $p = .007$, NSCLC adenocarcinoma $p = .024$ – Table 2). *VEGFA* expression remained a factor independently associated with *TP53* mutation in breast and colon carcinoma in multivariate analysis models including other VEGF ligands (VEGFA, VEGFB and VEGFC) and VEGF receptors (FLT1, KDR and NRP1): $p < .001$, odds ratio (OR)[Confidence Interval (CI) 95%] = 2.10 [1.74–2.54] for breast carcinoma, and $p = .005$, OR[$CI_{95\%}$] = 1.48 [1.13–1.95] for colon adenocarcinoma – Supplementary Table S1, **Panels B and C**). In contrast, squamous NSCLC tumors demonstrated a markedly different biomarker expression profile, with significant decreases in *VEGFC, FLT1*, and *KDR* expression levels (*VEGFC* p = .030, *FLT1* p = .035, *KDR* p = .007 – Table 2), as well as no increase in *VEGFA* expression (in univariate analysis). However, upon multivariate analysis, no change in expression in any of these factors was observed (Supplementary Table S1**, panel E**). Glioblastoma showed no significant change in expression in any factor associated with *TP53* mutations (Table 2 and Supplementary Table S1, **panel F**).

Similar results were found when considering the combined tumor set (pan-cancer analysis): angiogenesis ligands demonstrated significant increases of expression in *TP53*-mutated tumors, compared to their wild-type counterparts (multivariate analysis, *VEGFA* p < .001, *VEGFB* p = .007, *VEGFC* p = .012 – Table 2). Conversely, angiogenesis receptors demonstrated significant decreases of expression in *TP53*-mutated tumors (multivariate analysis, *FLT1* p = .049, *KDR* p < .001 – Table 2).

Table 1. Description of the pan-cancer cohort (N = 7,525 samples with known *TP53* status).

		Total samples	*TP53*-mutated samples
		N	N (% of subtype)
All cancer cohorts		7,525	2,670 (35%)
Adrenocortical carcinoma		52	12 (23%)
Bladder Urothelial Carcinoma		388	193 (50%)
Breast Invasive Carcinoma		960	292 (30%)
Cervical Squamous Cell Carcinoma & Endocervical Adenocarcinoma		190	9 (5%)
Cholangiocarcinoma		35	5 (14%)
Colon Adenocarcinoma		360	227 (63%)
Lymphoid Neoplasm Diffuse Large B-cell Lymphoma		41	5 (12%)
Glioblastoma Multiforme		136	44 (32%)
Head and Neck Squamous Cell Carcinoma		488	343 (70%)
Kidney Chromophobe		66	22 (33%)
Kidney Renal Clear Cell Carcinoma		431	9 (2%)
Kidney Renal Papillary Cell Carcinoma		161	5 (3%)
Acute Myeloid Leukemia		160	13 (8%)
Brain Lower Grade Glioma		509	247 (49%)
Liver Hepatocellular Carcinoma		190	60 (32%)
Non-small Cell Lung Adenocarcinoma		487	253 (52%)
Non-small Cell Lung Squamous Cell Carcinoma		178	145 (81%)
Ovarian Serous Adenocarcinoma		245	210 (86%)
Pancreatic Adenocarcinoma		113	72 (64%)
Pheochromocytoma and Paraganglioma		161	1 (1%)
Prostate Adenocarcinoma		332	41 (12%)
Rectum Adenocarcinoma		119	88 (74%)
Sarcoma		239	80 (33%)
Melanoma	Cutaneous	294	48 (16%)
	Uveal	80	0 (0%)
Stomach Adenocarcinoma		269	123 (46%)
Testicular Germ Cell Tumors		147	2 (1%)
Thyroid Carcinoma		397	3 (1%)
Uterine Corpus Endometrial Carcinoma		241	68 (28%)
Uterine Carcinosarcoma		56	50 (89%)

Abbreviations: N = number.

Table 2. Angiogenesis factors associated with TP53 mutations in selected and non-selected cancer cohorts.

		Univariate						Multivariate**	
				Non-small cell lung carcinoma				Pan-cancer	
		Breast carcinoma	Colon carcinoma	Adenocarcinoma	Squamous carcinoma	Glioblastoma	Pan-cancer	OR [95% CI]	P-value
Number of TP53-mutated samples (N)		*292*	*227*	*253*	*145*	*44*	*2,670*	*2,670*	
LIGANDS	*VEGFA* expression	Increased $p < .001$	Increased $p = .007$	Increased $p = .024$	-	-	Increased $p < .001$	1.16 [1.10–1.22]	$p < .001$
	VEGFB expression	-	-	-	-	-	Increased $p < .001$	1.07 [1.02–1.28]	$p = .007$
	VEGFC expression	-	-	-	Decreased $p = .030$	-	Increased $p = .038$	1.07 [1.01–1.12]	$p = .012$
RECEPTORS	*FLT1 [VEGFR1]* expression	-	-	-	Decreased $p = .035$	-	Decreased $p < .001$	0.93 [0.86–1.00]	$p = .049$
	KDR [VEGFR2] expression	Decreased $p = .003$	-	-	Decreased $p = .007$	-	Decreased $p < .001$	0.86 [0.80–0.93]	$p < .001$
	NRP1 expression*	-	-	-	-	-	Decreased $p < .001$	-	-

7,525 tumors presenting a *TP53* mutation were assessed – tumors with a *TP53* mutation were compared to those without a TP53 mutation, within individual cohorts. All *p*-values ≤.05 were considered significant (Mann Whitney U Test) and are presented.
*NRP1 is a co-receptor interacting with VEGFR1 and VEGFR2.
**p-values ≤0.1 in univariate were selected for multivariate analysis.
Detailed calculations are given in **Supplementary Table S1**.
Abbreviations: "-" = non-significant change; 95% CI = 95% confidence interval; N = number; OR = odds ratio.

TP53 mutation status remains independently associated with *VEGFA* expression in several multivariate models

To investigate the potential confounding effect of additional factors on *VEGFA* expression, we conducted three follow-up multivariate analyses using *VEGFA* expression as the dependent variable (i.e. the outcome whose variation is being studied) (Table 3). Each statistical model included *TP53* mutation status, with additional criteria such as tumor type (adenocarcinoma or squamous cell carcinoma), tumor localization, or mRNA expression of biomarkers related to hypoxia-mediated angiogenesis – hypoxia-induced factor 1 subunit α (*HIF1A*) and mouse double-minute 2 (*MDM2*).[21]

In each multivariate, *TP53* mutation status appeared independently and significantly associated with an increase in *VEGFA* expression ($p = .022$ OR[$CI_{95\%}$] = 1.26 [1.04–1.54], $p = .009$ OR[$CI_{95\%}$] = 1.31 [1.07–1.60], $p = .009$ OR[$CI_{95\%}$] = 1.30 [1.07–1.92] – Table 3). This relationship was preserved even in the presence of *HIF1A* and *MDM2* overexpression.

Outside of *TP53* mutation status, NSCLC squamous status also demonstrated a relationship with *VEGFA* expression, albeit a negative one ($p = .030$ OR[$CI_{95\%}$] = 0.33 [0.12–0.90], $p = .031$ OR[$CI_{95\%}$] = 0.33 [0.12–0.90] – Table 3), independent from *TP53* mutation status.

Both relationships were preserved when *VEGFA* expression was evaluated as a continuous variable instead of

Table 3. Analysis of factors associated with *VEGFA* mRNA expression in the pan-cancer cohort (N = 7,525 samples).

	VEGFA expression (categorical variable)*				*VEGFA* expression (continuous variable)	
	Univariate		Multivariate**		Univariate	Multivariate
	OR [95% CI]	P-value	OR [95% CI]	P-value	P-value	P-value
TP53 mutation	1.26 [1.04–1.54]	.022	1.26 [1.04–1.54]	.022	<.001	<.001
Adenocarcinoma	0.90 [0.74–1.10]	.313	-	-	.448	-
Squamous	1.06 [0.78–1.44]	.690	-	-	.009	-
TP53 mutation	1.26 [1.04–1.54]	.022	1.31 [1.07–1.60]	.009	<.001	<.001
Breast carcinoma	0.84 [0.61–1.14]	.296	-	-	.288	-
Colon adenocarcinoma	0.61 [0.35–1.07]	.101	-	-	.185	-
NSCLC adenocarcinoma	1.49 [1.06–2.10]	.026	-	-	.240	-
NSCLC squamous	0.38 [0.14–1.02]	.047	0.33 [0.12–0.90]	.031	<.001	<.001
Glioblastoma	1.04 [0.50–2.13]	.852	-	-	.074	-
TP53 mutation	1.26 [1.04–1.54]	.022	1.30 [1.07–1.59]	.009	<.001	<.001
HIF1A overexpression	2.01 [1.37–2.96]	.001	2.01 [1.36–2.95]	<.001	.044	.002
MDM2 overexpression	1.50 [0.96–2.34]	.081	-	-	.609	-
Breast carcinoma	0.84 [0.61–1.14]	.296	-	-	.288	-
Colon adenocarcinoma	0.61 [0.35–1.07]	.101	-	-	.185	-
NSCLC adenocarcinoma	1.49 [1.06–2.20]	.026	-	-	.240	-
NSCLC squamous	0.38 [0.14–1.02]	.047	0.33 [0.12–0.90]	.031	<.001	<.001
Glioblastoma	1.04 [0.50–2.13]	.852	-	-	.074	-

* *VEGFA, HIF1A, MDM2* over-expression were first considered as categorical variables, defined: **yes** if Z-score for mRNA expression ≥ 1.645 (i.e. biomarker is significantly overexpressed, compared to pan-cancer expression levels); **no** if Z-score for mRNA expression <1.645 (i.e. biomarker is not significantly overexpressed, compared to pan-cancer expression levels).
** Several multivariate models were built, selecting independent variables with *p*-value≤0.1 from univariate analysis. These include *TP53* mutation status, cancer type (adenocarcinoma vs squamous), selected cancer cohorts (breast, colon, lung and brain tumors), and *HIF1A* and *MDM2* over-expression.
Abbreviations: "-" = non-significant change; 95% CI = 95% confidence interval; NSCLC = non-small cell lung cancer; OR = odds rate

a dichotomized categorical variable (*TP53* mutation $p < .001$; NSCLC squamous status $p < .001$ – Table 3).

Relationship between specific TP53 mutation hotspots and VEGF-A expression

We performed a multi-comparison analysis considering different *TP53* mutational hostpots such as variants encompassing codons 175, 220, 245, 248, 273 and all other loci.

The level of *VEGFA* mRNA expression was not significantly different between each of these hotspot mutations (non-parametric ANOVA – Kruskal-Wallis test, $p = .903$, all individual comparisons were non-significant), suggesting that the association between *TP53* mutation and *VEGFA* expression is independent of the type of variant presented by the tumor.

Discussion

We provide evidence that somatic *TP53* mutation – one of the most frequent genomic alterations found in human tumors – is associated with an increase in expression of *VEGFA*, the major ligand of the VEGF/VEGFR pathway and a key regulator of angiogenesis.[22] This association, found in a pan-cancer analysis and in selected histological subsets, remains independent from other investigated factors and was preserved in a bidirectional fashion (i.e. *TP53*-mutated samples are enriched for *VEGFA* overexpression and *VEGFA* overexpressed tumors are more likely to present a *TP53* mutation, when considering different confounder variables) (Tables 2 and 3). As VEGF-A and its downstream receptors – FLT1 (VEGFR1) and KDR (VEGFR2) – are the targets of multiple FDA-approved therapies, demonstrating a relationship between *TP53* mutation and *VEGFA* expression may help to explain the correlation between *TP53* mutations and favorable outcomes observed in clinical studies of patients treated with anti-angiogenesis agents.[17–19] Though previously published data on the predictive value of circulating VEGF-A did not show a correlation with patient outcome after bevacizumab treatment, it is also known that blood-derived VEGF-A levels do not correlate well with tissue VEGF-A expression.[23,24]

Interestingly, the relationship between VEGF-A expression and mutant *TP53* was found in adenocarcinomas, but not in squamous cell cancers (Supplementary Table S2 and Table 1). This distinction has been previously described in NSCLC.[16] In this study, the association between *TP53* mutation and *VEGFA* expression was established in breast, colon and lung adenocarcinoma, but not in glioblastoma. Furthermore, lung squamous cell carcinomas did not present upregulation of VEGF-A and actually demonstrated downregulation of both receptors VEGFR1 and VEGFR2 (Table 2). VEGFR2 was downregulated across squamous cell cancers regardless of their origin (Supplementary Table S2).

TP53 mutation is a common molecular alteration (35% of all cancers in our study – Table 1) and is found across diverse malignancies, with particularly high mutation rates observed in certain tumor types. High-grade serous ovarian cancers, over 85% of which harbor *TP53* mutations, are unusual in that they respond positively to bevacizumab monotherapy.[25] Anti-angiogenic therapies are also important in fields of medicine outside of oncology. For instance, bevacizumab is effective in the treatment and prevention of recurrent pterygium, a benign vascular growth of the eye. Of interest, both *TP53* mutations and overexpression of VEGF ligands have been observed in this condition.[26–28] Additionally, several pre-clinical studies have shown mechanistic associations between *TP53* mutations and angiogenesis – in the presence of *TP53* mutations, HIF-1α, which is a transcriptional activator of VEGF-A, is increased.[8] Furthermore, transfected mutant *TP53* increases VEGF-A levels in bone marrow stromal cells.[11] In regard to HIF-1α, however, our observations suggested that VEGF-A and HIF-1α transcripts are both overexpressed in *TP53* mutated tumors, but the higher levels are independently associated with the *TP53* mutations (Table 3). The regulation of HIF-1α is mostly at the protein level; however, we did not find a significant association between the presence of *TP53* mutation and HIF-1a protein signal detection obtained by reverse-phase protein assay (RPPA) from The Cancer Genome Atlas (TCGA) (mean of protein expression of HIF-1a [95%CI] = 0.20 [−0.46–0.86] vs 0.49 [0.42–0.56], (*TP53* mutated versus not) $p = .317$); nor a significant association between VEGFA mRNA overexpression and HIF-1a protein level (mean of HIF-1a protein level [95%CI] = 0.38 [0.15–0.61] vs 0.49 [0.41–0.57], (VEGF-A mRNA high versus low) $p = .350$).

There are limitations to this study. For instance, this study focuses on *TP53* mutational status and does not assess other genomic variants. However, a more comprehensive study could possibly highlight additional molecular biomarkers significantly associated with elevated VEGF pathway expression and merits future investigation. Another limitation is that *TP53* mutation is being used to predict the expression of another biomarker (VEGF in this case). VEGFA expression or VEGF pathway expression could serve as a biomarker itself, but examining biomarkers based on protein expression (such as by immunohistochemistry) has produced variable results at times. Still, this concept should be explored. Another limitation is that we cannot know, based on the available data, if the *TP53* mutations are heterozygous or homozygous. In addition, we cannot state for certain that increased VEGF expression explicitly translates to increased angiogenesis. Another limitation is that many other genes in addition to VEGF are involved in angiogenesis and future studies should address the relationship between this complex network of genes and TP53. Finally, categorization of tumors into adenocarcinomas and squamous cancers across multiple sites of origin represents an oversimplification; however, this represents an attempt to further stratify the analyses completed. Significant further investigation is required to truly elucidate if increased VEGFA mRNA expression in p53-mutated tumors is preserved across multiple types of adenocarcinomas.

Based on this data and the above limitations, it cannot be assumed that all TP53-mutant cancers will respond to anti-angiogenesis agents or that TP53 wild-type tumors will not respond; rather the response rates are expected to be higher in TP53-mutant tumors, consistent with prior studies in the literature.[17–19]

In summary, using a large pan-cancer cohort of 7,525 samples, we demonstrate that increased VEGF-A transcript levels correlate independently with tumor *TP53* mutation status, particularly in adenocarcinomas, independent of their

organ of origin. This observation may provide a further mechanistic underpinning for the association between *TP53* mutations and response to anti-angiogenesis agents and may be of great interest when one considers the high frequency of deleterious *TP53* genomic events in human tumors.

Materials and methods

Molecular data retrieval: Data corresponding to 7,525 unique tumor samples, representing 30 different tumor cohorts, were retrieved from the TCGA database (https://cancergenome.nih.gov/): sequencing-based *TP53* mutation status and mRNA expression Z-scores (indicating the number of standard deviations away from the mean expression level of the population) of angiogenesis ligands *VEGFA, VEGFB*, and *VEGFC*; receptors FMS-like Tyrosine Kinase-1 (*FLT1* or *VEGFR1)* and Kinase Insert Domain Receptor (*KDR or VEGFR2)*; and co-receptor Neuropilin-1 (*NRP1*, co-receptor to *FLT1* and *KDR*) (Supplementary Figure S1).

***TP53* mutation and *VEGF* biomarker expression association analyses**: We analyzed the association between *TP53* mutation status and mRNA expression levels of the genes listed above. This analysis was carried out for the entire tumor set as well as for common cancer cohorts in which bevacizumab is commonly used – glioblastoma, NSCLC, and colon cancer. Breast cancer was also included due to the availability of a large number of samples and because bevacizumab was formerly indicated for this disease. We further analyzed the relative associations between factors such as *TP53*-mutation status, cancer cohorts, cancer subtypes, and *VEGFA* expression (multivariate analysis). *VEGFA* expression was characterized as a continuous variable and also dichotomized as a categorical variable, defining mRNA expression Z-scores ≥1.645 as "overexpression" of *VEGFA*. A Z-score ≥1.645 corresponds to a value in the top 5% of biomarker expression, conferring significance considering a one-tailed p-value of 0.05.

Statistical analysis: Statistical analyses were performed by AB, using SAS University Edition. All univariate analyses were done using a Mann-Whitney univariate test or Fisher's exact test. Parameters with a p-value (p)≤0.1 in univariate analysis were selected for follow-up multivariate analyses, in order to further describe the factors independently associated with TP53 mutation or VEGFA overexpression. *P*-values ≤0.05 were considered statistically significant.

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